two cohorts by incorporating the resulting coefficients of multivariable Cox regression model into a score that utilizes linear gene expression values. This gene expression classifier was validated in 6 additional publicly available datasets of stage I/II lung SCC (N = 358). The classifier identified high-risk patients in multiple large-scale and geographically diverse cohorts (N = 570). The results appear to be independent of race and gene expression platform. Canonical pathways associated with this signature encompass proteins involved in signal transduction, tissue remodeling, and cell motility that would broadly lead to cancer cell migration, invasion and proliferation, suggesting that the gene signature identifies molecular subsets of patients with clinical relevance. This gene classifier could be used to guide clinical decisions after surgical resection. Thus, we would advocate that this classifier be incorporated into prospective trials for further evaluation of its clinical effectiveness.

A functional SNP in MRPL43 modulates lung cancer susceptibility and survival through alternative splicing of its isoforms

Andrew Mcclary,[1] Kara Calhoun,[1] Jessica Roberts,[1] Ana Robles,[1] Elise Eowman,[1] Majda Haznadar,[1] Dominic Esposito,[2] Cassidy Alexandre,[1] Mohammed Khan,[1] Rama Modali,[3] Hirokazu Okayama,[4] Kouya Shiraishi,[4] Yun Yokota,[4] Takashi Kohno,[4] Brid Ryan[1] *[1]Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, [2]Frederick National Laboratory, NCI, Frederick, MD, [3]Bioserve Ltd, Gaithersburg, MD, [4]Division of Multistep Carcinogenesis, National Cancer Center Research Institute, Tokyo, Japan*

miRNAs, a class of non-coding genes, modulate mRNA translation and stability by primarily binding to the 3'UTR of mRNA transcripts. Genetic variation within a miRNA gene could modulate the thermodynamic interaction between the miRNA and its target mRNA sequence and modulate expression. Many studies have demonstrated the importance of miRNAs in cancer biology, including lung cancer. Indeed, we recently published several studies showing the relationship between functional SNPs in microRNA binding sites and the relationship with lung cancer risk and outcome. However, few studies that systematically examined SNPs in miRNA genes and their relevance to lung cancer have been conducted to date to our knowledge. We conducted a genome-wide analysis of SNPs in microRNA genes and assessed their relevance to human lung cancer. Using samples from the NCI-MD study, we examined 22 SNPs (after selections procedures) in 974 European Americans and found that rs4919510 (Chr10 q24.31) in miR-608 was associated with a 2.55 fold increased risk of lung cancer, after adjustment for age, gender, pack-years of smoking and smoking status (OR 2.55 95% C.I. 1.04-6.28; P=0.041). The G allele of rs4910510 is common; the MAF is 18%, 57% and 53% in European Americans, African Americans and Japanese, respectively. We therefore tested for population conversion of this epidemiological observation in 2 other geographical populations, i.e., an African American population and a Japanese population. In both studies, the G allele of rs4919510 was associated with an increased risk of lung cancer (African American: OR 2.73, 95% C.I. 1.44-5.14; P=0.002; n=566) (Japanese: OR 1.85, 95% C.I. 1.18-2.86; P=0.007; n=768). We also found that rs4910510 was associated with lung cancer survival. The GG genotype of rs4919510 was significantly associated with prolonged survival among European Americans (HR 0.15, 95% C.I. 0.04-0.62; P=0.008) and Japanese patients (HR 0.66, 95% C.I. 0.45-0.97; P=0.036), while a similar trend was observed in African Americans (HR 0.64, 95% C.I. 0.35-1.16; P=0.1). An eQTL analysis using samples from the NCI-MD study found that the SNP did not affect miR-608 processing and functional studies comparing the function of miR-608-C and with miR-608G did not reveal any differences in either mRNA targeting or function. We subsequently mapped the functional significance of the rs4919510 association to a neighboring gene, MRPL43, which is a component of the mitochondrial ribosome. Our results show the miR-608 SNP is actually in linkage with a SNP in MRPL43 that creates a donor splice site at the exon 3/intron 3 boundary. Thus, rs4919510 is a tag allele for the real functional locus in MRPL43. The SNP changes the splicing pattern of MRPL43, whereby expression of the short and predominant isoform is decreased, and the longer isoforms is increased (P<0.0001). We replicated this observation in two different lung cancer tissue sets and also 6 other tissue types using TCGA data (all P<00001) and also across populations of different ancestry. We further found that the SNP modulates mitochondrial metabolism by altering the balance between oxidative phosphorylation and glycolysis. Our initial study aimed to evaluate the association between microRNA SNPs and lung cancer. In summary, we did not observe a strong relationship between miRNA SNPs with either lung cancer risk or survival. However, we have identified a SNP in MRPL43, a subunit of the mitochondrial ribosome, which is associated with risk

and survival in lung cancer patients. The SNP, which arose in Africa, modulates the splicing of MRPL43, leading to a linear decrease in the major isoform and a switch in the balance of oxidative phosphorylation and glycolysis. Our work identifies a new pathway of potential relevance to lung cancer and provides new insight into the role of mitochondrial ribosomes in cancer.

Prognostic nomogram for predicting survival of non-small-cell lung cancer patients*

Jeong Seon Ryu, Bo-Rim Yi, Jung Soo Kim *Inha University Hospital, Incheon, Incheon, Korea*

A few prognostic factors have been widely accepted in non-small-cell lung cancer (NSCLC). Nomogram is a useful tool of providing more accurate prognostic information in cancer patients. For the first time, we tried to develop prognostic nomogram for patients with stage III/IV non-small-cell lung cancer (NSCLC) who seek medical care in daily clinical practices.

Using the 15 pretreatment readily available clinical variables in 752 patients with stage III/IV NSCLC, we performed a Cox proportional hazard analysis with hierarchical backward elimination methods to identify survival prognostic factors. Then using these independent prognostic factors, the prognostic nomogram was constructed from data collected from a test group of 376 (50%) randomly sampled patients with NSCLC and it was validated with data from the remaining 50% of the patients.

The independent poor prognostic factors that were included in the nomogram are as follows: male, weight loss of more than 5%, Eastern Cooperative Oncology Group performance status greater than 1, histology of no squamous cell carcinoma, Tumor-Node-Metastasis stage greater than IIIA, increased serum calcium, low serum hemoglobin, and no chemotherapy. The prognostic nomogram constructed from eight independent prognostic factors predicted survival well with a concordance index of 0.66, which was validated by an internal group of patients.

We developed a unique prognostic nomogram constructed using pretreatment readily available clinical variables to predict the probability of survival in NSCLC patients. This prognostic nomogram may help researchers in designing randomized clinical trials as well as assist clinicians in selecting the most appropriate therapy for NSCLC patients.

**Due to unforeseen circumstance, this poster was not presented.*

High expression of endoplasmic reticulum oxidoreductin (ERO) 1L is associated with resistance to cisplatin

Toshio Sakatani, Keita Maemura, Noriko Hiyama, Yousuke Amano, Kousuke Watanabe, Hidenori Kage, Masashi Fukayama, Jun Nakajima, Yutaka Yatomi, Takahide Nagase, Daiya Takai *The University of Tokyo, Bunkyo, Tokyo, Japan*

Various conditions such as low oxygen and low nutrition cause accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), leading to ER stress. To overcome this, cells have resistance mechanisms called unfolded protein response (UPR). When UPR is insufficient, ER-mediated apoptosis occurs. Recent reports have shown that several diseases including cancers can be caused by ER stress. In cancers, high proliferation rates and the presence of mutated gene products lead to accumulation of unfolded and misfolded proteins in the ER and adaptation to ER stress is essential for survival of cancer cells.

As preliminary analysis, we used publicly available expression profiles (GSE10245 and normal tissue data sets) and analyzed expression levels of 84 ER stress pathway genes. We found ER oxidoreductin 1L (ERO1L) was highly expressed in many types of cancer tissue, while expression was low in normal tissues. In addition, using Oncomine™, we found ERO1L high expression was associated with poor prognosis of non-small cell lung cancer. There are some reports that expression of ERO1L is beneficial to tumor cells, but association with lung cancer has not been reported. Therefore , we sought to determine the role of ERO1L in lung cancer, including response to therapy.

Using quantitative RT-PCR and Western blotting, we identified NCI-H441, HCC2935, and NCI-H2347 as ERO1L high expression cell lines. NCI-H520, NCI-H522, and SK-LU-1 were ERO1L low expression cell lines. We then exposed these cells to cisplatin (CDDP) and determined the half maximal inhibitory concentration (IC50). IC50 values for CDDP was 91, 64, and 21 μM, respectively, in cells with high ERO1L expression. IC50 values for CDDP was 2.6, 4.1, and 22 μM, respectively, in cells with low ERO1L expression. We then exposed cell lines to both CDDP and EN460, an ERO1L inhibitor. When CDDP was combined with low dose EN460 (1/10 of IC50), there was a trend toward decreased IC50 for CDDP. We also evaluated IC50 values for CDDP plus high dose EN460 (1/3 of